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The Metabolic Influence of Copious Water Drinking

Chemical Engineering

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THE METABOLIC INFLUENCE OF COPIOUS WATER DRINKING

BY

CHESTER C. FOWLER

THESIS FOR THE DEGREE OF BACHELOR OF SCIENCE

IN CHEMICAL ENGINEERING

IN THE

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OF THE

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June 1st 1909

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Chester Charles Fowler.

ENTITLED The Metabolic Influence of Copious Water Drinking.

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Bachelor of Science in Chemical Engineering.

R. B. Hawk.

Instructor in Charge.

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Introduction.

As far back as the year 1852 we find references on the effects of copious water drinking. In that year Bidder and Schmidt found, in various experiments, that there was a very small increase in the excretion of nitrogen following increased water ingestion. Since that time there have been a large number of investigators at work on different phases of this metabolic problem. These investigators may be divided into three groups for general classification: First, those who found a very small increase, or an entirely negligible increase in the total output of urinary nitrogen; second, those who found an increase in nitrogen out of all proportion to the increase in water ingested; and third, those whose results seemed to give conflicting data.

To the first group belong Munk[25], Feder[18], Flack[19], Panum[14], Voit[6], E. Salkowski[17], Strohmann[26], Rost and Frühling[26], Weiske[27], Kennepohl[27], Schulze[27], Bartels[28], Smith[8], Meyer[23], Winternitz[9], Mosler[49], Edsall[48], Rosemann[45], Böcher[36], Kaupp[4], Bidder and Schmidt[1], and TerGrigorianz[29], the first six of whom report experiments performed on normally fed animals, generally dogs. Weiske, Kennepohl and Schulze worked with sheep and Strohmann, Rost and Frühling used as subjects goats. The rest of the experimenters report data collected from various orders of experiments but all tending to point to the same general result.

Voit noticed that metabolic processes were facilitated, when an increase in the amount of water ingested was made, protein katabolism was stimulated and the volume of the urine increased. He found that the excretion of nitrogen was increased by giving an increase of water to a fasting dog.

Panum feeding dogs on a uniform diet noticed that fifty per cent of the urea for a daily period was excreted in the first eleven hours. If , for a period of three days the sebjects were given no water, the increase in the excretion of urea following the taking of food, ceased after from one to four hours; if under the same conditions and twelve hours after the meal, when the urea excretion was probably uniform, 500 c.c. of water was given, a slight increase in the urea excretion was noticed.Thus we see that an increase in the urea excretion follows when water is taken after the rate of the urea output has become uniform.

E.Salkowski reports, in a set of experiments upon normally fed dogs, that:the increase in the elimination of urinary nitrogen, upon increasing the amount of water ingested, was very small or entirely negligible. Feder in his work along this line reports that on doubling the water ingested, after first having brought the subject to nitrogen equilibrium, the urea excretion is increased four grams the first day and then drops off below normal on the second day although the increased water ingestion was maintained. The rest of the observers in this group have obtained practically the same data, and all show this slight increase in the nitrogen excretion on increasing the water ingested.

It might be well here to note the theory advanced by Meyer in reference to this increase of nitrogen excretion. He concluded that, on an increased consumption of water the urea and other more highly organized nitrogen-containing bodies are washed out of the tissues and thus the urine nitrogen-content raised. He claims however that an increase in the excretion of nitrogen, and a large volume of urine, do not necessarily go hand in hand. Weiske, studying the influence of sodium chloride and water, found the excretion of

nitrogen to vary directly with the quantities of sodium chloride and water ingested; while Ter-Grigorianz working upon human subjects concluded that water aids protein katabolism.

To the second group belong Becher[3], Rubner[30], Forster[22], Salkowski[37], Eichorst[38], Schondorff[35], Genth[5], Weigelin[11], Bischoff[2], and Foster[51]. Becher found that a period of several days was necessary to regain the normal excretion of nitrogen, after the increased output of nitrogen following an increase in the ingestion of water. Rubner found, that the excretion of urea was twice as great when fasting rabbits were given water ad libitum, as compared with the output of the same rabbits when these animals were deprived of water. Forster on adding 1828 c.c. of water to the normal water taken by a dog got an increase of ten grams of urea. The increase from 1.8 grams one day to 8.5 grams the next day by adding 800 c.c. of water to the constant diet of a dog is the result observed by Eichorst. In 1856 Genth in experimenting on himself found a great increase in the excretion of urea by drinking large quantities of water. So also Salkowski, Schöndorff, Weigelin and Bischoff all contribute data pointing to the fact that the ingestion of water influences the excretion of nitrogen by the urine.

The third class, which we have mentioned in connection with this history of the researches on the effects of copious ingestion of water on the different phases of nitrogen metabolism, consists of Henneberg[7], Oppenheim[24], Neumann[50], Dubelir[34], Seegen[12], Fränkel[20] and Roux[16]. Henneberg came to the conclusion, that on increasing the water ingested, an apparently permanent increase in nitrogen excretion occurs. But again we find Oppenheim and Neumann taking the opposite stand. Oppenheim used a uniform normal diet and when approximate nitrogen equilibrium was reached four liters of water

was added to the normal diet; two liters being taken at 1 P.M., one liter at 5 P.M. and the remaining liter of water at 9 P.M. Following the ingestion of the two liters of water there occured an increase of six grams above normal in the urea excretion, but during the next four hours although an additional liter of water was ingested the excretion fell below normal with only a very slight increase after the ingestion of the fourth liter of water. These results bear a striking resemblance to those which Feder obtained and which have been mentioned in this review under group one. Oppenheim concludes that the increase is only temporary and that the excess of nitrogen is only due to a washing of urea from the tissues and that this urea which would have appeared normally but at a later time and more gradually.

Neumann made a set of experiments in which he was the subject and studied the effect of increased and decreased water ingestion. Three liters of water caused, on the first day, an excretion of 15.9 grams of nitrogen an amount exceeding that excreted on the last day of the normal period by 3.81 grams. On the three following days the excretion gradually fell off until on the third day 12.2 grams only was excreted. He found that a decrease in water ingestion caused a corresponding falling off of the nitrogen output and that small additional amounts of water caused small rises. This experiment lasted throughout a period of twenty-four days and was divided into five periods. The last period he devotes to the study of the effects of decreased amounts of water and he makes no attempt to bring himself back to nitrogen equilibrium on his initial uniform diet.

Dubelir, in studying the effects of increased water ingestion on a small male dog, came to the conclusion that no increase in nitrogen excretion was secured. Seegen obtained results which support the view taken by Dubelir. He made an expreiment of fifty six days duration and varied the water ingested from 500 c.c. to 1800 c.c. per day. But nevertheless he found that forty-one grams of nitrogen was excreted alike on days of large and small water ingestion.

Again we find two more investigators Fränkel and Roux who procured data pointing toward the constancy of the nitrogen output. Fränkel, by allowing a fasting dog 1240 grams of water at one time, noticed no effect in the excretion of urea. Roux even goes still farther and we find in his data that on increasing the water from 925 grams to 2515 grams there was a fall of 1.1grams in the daily urea output.

In 1905 there appeared in the University of Pennsylvania Medical Bulletin a series of three experiments by P.B.Hawk,[53] in which five individuals were used as subjects, there being one subject in the first experiment and two in each of the other two. The first subject drank 4500 c.c. additional water in the water period and the subjects of the other two experiments each took 4000 c.c. additional. This water was taken in definite quantities at different intervals in each experiment. In each case the author noted a marked increase in the nitrogen excretion at the beginning of each water period which is followed by a gradual falling off until toward the end of the period approximate nitrogen equilibrium is reached. Hawk concludes that the increased nitrogen was due primarily to the removal from the tissues of nitrogenous decomposition products which had accumulated there; and, secondly to an

increased protein catabolism.

In addition to the investigators already mentioned we might refer to Karchagin[21] who studied the effects of diminished water ingestion. He found that the body weight fell and from his data concluded that assimilation of nitrogen was somewhat improved while the metabolism of nitrogen was decreased slightly. Speck,[10] Gley and Richet[31], J.W.Paton[13], Landauer[44], Straub[47] and VonNoorden[40] have all given us points of varying interest on the effect of the copius ingestion of water.

There has been considerable work done concerning the effects of mineral-waters on the metabolism of nitrogen. These, however, have no direct bearing upon our investigation and will simply be mentioned here. The principal experimenters along this line have been Navasartianz[39], Julius Katz[41], J.Leva[42], Ludwig, Bain and Edgecomb[52], and Van de Weyer and Wybauw.[54]

Purpose of the Experiment.

Altho there have been a large number of investigations made upon the influence of copious water drinking, yet nowhere in so far as we have been able to discover from a careful study of the literature, has there been a carefully conducted experiment or series of experiments made upon human subjects with the end to find out the effect of the copious ingestion of water with the meals, and a study made of the ammonia, urea, nitrogen, creatinine, and creatine eliminations. The greater number of experiments along this line were concerned solely with the excretion of total nitrogen by the urine and in a large percentage of the experiments no analysis of the foods or feces was thought necessary. Also no attempt was made to get the individual into nitrogen equilibrium before the

experiment proper was begun. Hence we have in many of these experiments, metabolism data which have very little practical value.

Still other investigators used accurate methods for the determination of total nitrogen excretion by the urine, and were careful to get their subjects into a condition of approximate equilibrium but neglected to make analyses of the feces. Obviously not knowing the proportions of nitrogen represented by the feces such experiments would not permit of the drawing of any definite conclusions regarding the relation between the income and outgo of nitrogen during any period.

In the experiment we are about to report the subject was placed on a normal, constant diet and by means of a preliminary period of sufficient length, he was brought to a condition of approximate nitrogen equilibrium. At that point 1000 c.c. of water was added to each meal, and continued thus through a period of five days. Immediately following this period came a final period during which the original normal constant diet was again maintained and the subject brought back to a condition of nitrogen equilibrium.

The urine was collected in twenty-four-hour samples while the feces was collected in period samples. The foods, with the exception of milk, were analysed before the experiment began after first preparing a satisfactory sample of each of the foods to be used. The milk was analyzed at frequent intervals during the experiment. Thus accurate knowledge of the income and outgo of nitrogen was obtained and conclusions made possible.

Description of Experiment.

Subject and Daily Schedule. The subject of this experiment was a young man twenty-two years of age and weighing at time of commencement of the experiment 71.69 kilograms.

The daily schedule for the preliminary and final periods of the experiment was as follows:

6:30 A.M. Arose, defecated, weighed.
7:30 A.M. Breakfasted (100 c.c. water with meal).
10:00 A.M. 200 c.c. water taken.
12:30 P.M. Noon meal (100 c.c. water with meal).
3:30 P.M. 200 c.c. water taken.
6:15 P.M. Dinner (100 c.c. water with meal)
8:30 P.M. 200 c.c. water taken.

On the days of the increased water ingestion 1000 c.c. of water was added to each of the three daily constant meals. This additional water was to be drunk throughout the progress of the meal.

Diet: The articles of diet were butter, peanut-butter, whole milk, soda crackers, cornflakes, and sugar; 25 grams of butter, 20 grams of peanut-butter, 400 grams of whole milk, 100 grams of soda crackers, 25 grams of cornflakes, and 12 grams of sugar constituting the menu for one meal. Each meal of the day was absolutely identical with the others, and thus was procured an absolute uniformity of diet throughoutthe entire experiment.

Collection of Urine and Feces: The experimental day extended from 7 A.M. to 7A.M.If possible the subject defecated each morning before breakfast and then immediately determined his body weight. This schedule was followed carefully every day throughout

the experiment and hence it is obvious that each day was begun with the body in a very similar condition.

The feces was collected in period samples. To secure satisfactory differentiation of the feces of one period from the feces of the following period, a gelatin capsule containing about 0.2 gram of powdered charcoal was taken just before breakfast of the first day of the new period. This powdered charcoal formed a more or less regular black mass in the fecal matter and thus rendered the separation of the feces of the one period from the succeeding period a comparatively simple matter.

Methods of Analysis. Nitrogen: The method used by us for the determination of total nitrogen is one of the numerous modifications of the Kjeldahl Method. The principle of the method being the conversion of the various nitrogenous bodies of the urine, feces or foods into ammonium sulphate by boiling with concentrated sulphuric acid, the subsequent decomposition of the ammonium sulphate by sodium hydroxide and the collection of the liberated ammonia in an acid of known strength. This partly neutralized acid solution is finally titrated with an alkali of known strength and the nitrogen content of the sample calculated.

The procedure, in the case of urine, is as follows: Place 5c.c. of urine in a 500 c.c. long-necked, Jena Kjeldahl flask, add 20 c.c of concentrated sulphuric acid and about 0.2 gram of cupric sulphate and digest for one hour after the solution becomes colorless. Allow the flask to cool and dilute the contents with about 200 c.c. of water. Add a little more of a concentrated sodium hydroxide solution than is necessary to neutralize the sulphuric acid and introduce into the flask a little coarse pumice stone, to prevent bumping, and a small piece of paraffine to lesson the tendency to froth. By

means of a safety tube connect the flask with a condenser so arranged that the delivery tube passes into a vessel containing a known volume of tenth normal sulphuric acid, using care that the end of the delivery tube reaches beneath the surface of the fluid. Mix the contents of the distillation flask very thoroughly by shaking and distil the mixture until the volume has diminished about one half. Titrate the partly neutralized tenth normal acid, with tenth normal alkali, using congo red as indicator.

Ammonia: The method used for this determination was that of Folin. Twenty-five c.c. of urine are placed in a cylinder about 30 cm. high and about one gram of dry sodium carbonate is added also a little crude petroleum to prevent foaming. Insert into the neck of the cylinder a rubber stopper provided with two perforations into each of which passes a glass tube, one of which reaches below the surface of the liquid. The shorter tube (10 cm. in length) is connected with a calcium chloride tube filled with cotton and this tube is in turn joined to a glass tube extending to the bottom of a 500 c.c. wide-mouthed flask which is intended to absorb the ammonia and for this purpose should contain 20 c.c. of tenth normal sulphuric acid, 200 c.c. of distilled water and a few drops of "alizarin red" as indicator. To insure the complete absorption of the ammonia the absorption flasks were provided with Folin improved absorption tubes. In order to insure no error on account of the presence of ammonia in the air another absorption flask was fitted to the other side of the cylinder. This whole apparatus is attached to an ordinary filter pump and the air drawn through, entering the last named flask first, then through the cylinder and absorption flask.

In about one and one-half hours the last traces of ammonia should be removed . At this point the absorption flask is removed and the unneutralized acid determined by titration with tenth normal sodium hydroxide.

Urea: For this determination the Benedict and Gephart method was used. This method consists of the following manipulation. Five cubic centimeters of urine are introduced into a small Erlenmeyer flask along with an equal volume of (1:4) hydrochloric acid. The vessel is then placed in an autoclave and maintained at a temperature of 150° - 155° C. for one and one-half hours. After the autoclave has cooled the contents of the flask are washed into an 800 c.c. Kjeldahl flask and diluted to about 400 c.c. with distilled water. Twenty cubic centimeters of ten per cent sodium hydroxide solution is added and then the mixture is distilled for forty minutes into a known excess of standard acid. The process is then continued as under "Nitrogen" above, and the original ammonia content as calculated under "ammonia" subtracted from total ammonia (ammonia plus urea) obtained here gives the urea content of the urine.

Creatinine : The only method upon which any degree of reliability can be put, for this determination and the one used by us in this experiment is Folin's colorimetric method. It is based upon the characteristic property possessed by creatinine alone, of yeilding a definite color reaction in the presence of picric acid in an alkaline solution. The exact nature of this reaction is a subject about which not very much is known.

The procedure is as follows: Place 10 c.c. of urine in a 500 c.c. volumetric flask, add 15 c.c. of saturated picric acid

solution and 5 c.c. of a ten per cent solution of sodium hydroxide. Shake thoroughly for half a minute and then allow the mixture to stand for five minutes. During this interval pour a little half normal potassium bichromate solution into one of the cylinders of a Duboscq colorimeter and carefully adjust the level of the corresponding prism to 8.0 m.m. . At the end of the five minute interval, the contents of the 500 c.c. flask are diluted to 500 c. c. and thoroughly mixed. Then using this solution the other cylinder of the colorimeter should be washed out and finally a little of the solution put in and adjusted as the other was except that this prism is raised and lowered until the two colors seen, correspond exactly, then the reading on the side corresponding to the urine should be recorded. As practice, both cylinders should first be filled with the bichromate solution, one prism adjusted at the 8.0 m.m. level and the other used as a check on the eye. At least four readings should be taken until the operator becomes thoroughly familiar with the manipulation.

The calculation is based on experimental evidence. It has been found that 10 mg. of pure creatinine when dissolved and diluted to 500 c.c. as above yield a mixture 8.1 m.m. of which possesses the same colorimetric value as 8.00 m.m. of a half normal solution of potassium bichromate. Hence suppose that y equals the number of m.m. of the unknown solution equivalent to 8.0 m.m. of the potassium bichromate . Then

y : 8.0 :: 10.0 ; x (mgs. of creatinine in quantity of urine used)

Creatine: The method of determination is the Folin-Benedict method and is as follows: To twenty cubic centimeters of urine,

in a 50 c.c. graduated flask, 10 c.c. of normal hydrochloric acid is added and then the flask and contents are placed in an autoclave and heated at a temperature of 117 - 120° C. for half an hour. Remove and cool the urine and then dilute up to 50 c.c. and shake. Of this volume take 25 c.c. place it in a 500 c.c. volumetric flask and proceed as with creatinine except that instead of using 5.0 c.c. of ten per cent sodium hydroxide with the picric acid use about twelve to fifteen cubic centimeters.

The calculation is made the same as for creatinine, the difference between the former reading and this reading giving the creatine content of the urine in terms of creatinine.

Moisture on Feces: In this determination about two grams of the thoroughly mixed moist composite sample of feces were weighed out by difference from a weighing tube into lead caps and then placed in the drying oven at 103° C. for seventy-two hours. At the end of this time the cap and contents were placed in a desicator and allowed to cool, when the dried feces and cap were weighed.

Bacteria in Feces: Strasberger's method was used in this determination and a detailed description of the manipulation may be found in "The Journal of Infectious Diseases" Volume 6, No.2, April 1, 1909, Page 129. (MacNeal, Latzer and Kerr).

Discussion of Results.

Excretion of Nitrogen: The nitrogen content of the urine for the fifth day of the preliminary period was 13.084 grams and for the sixth day was 13.183 grams. The elimination of nitrogen by the urine being so very uniform for these two days, thus signifying that a satisfactory plane had been reached and

that nitrogen equilibrium was fairly well established. On the following day, the seventh of the experiment, 3000 c.c. of water was added to the diet, 1000 c.c. being taken during the progress of each meal. The effect of this large addition to the amount of water taken was seen immediately not only in the increased volume of urine having a very low specific gravity, but also in the marked increase in the nitrogen excretion for the day. This large amount of water was taken, as we have seen, when the body was in a condition of approximate nitrogen equilibrium and excreting daily an average of 13.134 grams of nitrogen by the urine. By examination of Table II we see that this average excretion of nitrogen for the preliminary period was greatly increased by the drinking of 3000 c.c. of water additional and that the first day of the water period showed an excretion of 14.161 grams of nitrogen by the urine, an increase of 1.027 grams or 7.82 per cent (see Table IV), above the average elimination of the preliminary period. The excretion of nitrogen by the urine on the second day of the water period, while not so great as that of the first day, was still in excess of the average normal output of the preliminary period. Here, with the addition of 3000 c.c. of water to the constant diet and at meal times, we obtained an excretion of 13.491 grams of nitrogen, this being 0.357 gram above the average daily excretion for the preliminary period. On the next two days of the water period the nitrogen excretion by the urine fell below normal being 12.981 grams for the ninth day and 12.970 grams for the tenth day. On the eleventh day of the experiment however, the final day of the water period the excretion was found to be 13.138 grams which is approximately

the same as that of the last two days of the preliminary period. Hence we see, by referring to the data, that a daily addition of 3000 c.c. of water, to a constant diet, caused a total increase of 1.077 grams of nitrogen for the period (see Table V).

On the twelfth day of the experiment the final period of the investigation was begun. The diet of this day, as well as that of the seven succeeding days, was the same constant diet as that fed during the preliminary period. This diet on the first day of the new normal period gave an excretion of 11.823 grams of nitrogen as compared with the average daily excretion of 13.134 grams of nitrogen of the preliminary period. This showed a decided decrease in the excretion of nitrogen, being 1.311 gram less than the normal excretion of the preliminary period and 1.526 gram lower than the average excretion for the water period. On the thirteenth day there was a slight increase of 0.013 gram in the excretion, but on the fourteenth there occurred a rise of over one gram. On the last three days of the final period the excretion had practically returned to normal again, the seventeenth showing an excretion of 13.030, while the excretions of the eighteenth and nineteenth days were respectively 12.905 grams and 13.100 grams, the average of these three days giving us a value of 13.032 grams as compared to the average output of 13.134 grams per day for the last two days of the preliminary period. This indicates very clearly that the original condition of nitrogen equilibrium, which existed before the ingestion of the large volume of water, had been re-established. Therefore it is evident that the total effect upon the excretion of nitrogen by the urine, of the addition of fifteen liters of water to the diet during the meals throughout a period of five days, is

measured by taking into consideration all the daily excretions of nitrogen from the first day of the water period until the condition of nitrogen equilibrium was again established upon the seventeenth day of the experiment. By referring to Table II we see that the total increase in the excretion of nitrogen during the water period was 1.077 grams all practically occurring on the first day of increased water ingestion. Also we may see that in the final period before the subject was brought back to nitrogen equilibrium again, there was a total decrease in the excretion of nitrogen amounting to 5.123 grams of nitrogen. Now subtracting 1.077 grams from 5.123 grams we find that there was a gain of 4.046 grams of nitrogen to the body judging alone from the urinary data. (See later discussions of income and outgo, p.24.)

Excretion of Urea: In general the variations in the urea content of the urine followed very closely the variations in total nitrogen. The fifth and sixth days of the experiment showed excretions of urea nitrogen equal to 11.476 and 11.568 grams respectively(Table II), the average of these two days being 11.522 grams. On the first day of the water period there occurred an increase of 1.074 grams of urea nitrogen(see Table IV).The eighth experimental day showed a drop of 1.013 grams of urea nitrogen as compared to a drop of only 0.67 gram in the total nitrogen content of the urine. Thus the percentage of total nitrogen which was due to urea,(see Table III), decreased from 88.99 % on the first day of the water period to 82.17 % on the third day of the water period from which day to the end of the period there was an increasing percentage of the total nitrogen which was due to urea. It is a striking fact that on the final day of the water period 91.79 % of the total nitrogen appeared

in the form of urea, this being an increase of 3.19 per cent over the first day of the water period. On the first day of the final period the urea content of the urine fell and only 84.17 per cent of the total nitrogen of that day was urea nitrogen, a decrease of 8.30 per cent. On the second day of the final period 86.16 per cent of the total nitrogen was in the form of urea nitrogen, a value which was maintained with great uniformity for four days. On the sixth day of this period and the seventeenth of the experiment, however, only 83.16 per cent of the total nitrogen was in the form of urea nitrogen. This sudden fall was followed on the eighteenth and nineteenth days of the experiment by urea nitrogen values which were closely comparable to the urea excretion of the last portion of the preliminary period, the average for the two days being 87.27 per cent.

Excretion of Ammonia: The average excretion of ammonia nitrogen for the last two days of the preliminary period was 0.337 grams or 2.57 per cent of the total nitrogen. On the first day of the water period the ammonia content of the urine increased (see Table IV) and remained higher for the whole water period with the exception of the last day, when there was a slight drop of 0.061 gram below the normal preliminary excretion. The average daily increase in ammonia nitrogen for the water period was 0.233 gram or an increase above the ammonia nitrogen of the preliminary period of 68.8 per cent (see Table V). The average daily increase for the interval between the beginning of the water period and the time equilibrium was reestablished on the sixth day of the final period was 0.180 gram, or an increase of 53.6 per cent. On the three final days of the experiment the average

excretion of ammonia nitrogen was 3.73 per cent of the total nitrogen, which is only 0.16 per cent higher than the excretion of ammonia nitrogen for the preliminary period.

Excretion of Creatinine: On the first day of the water period there appeared a drop in the excretion of creatinine, amounting to 0.025 of a gram of creatinine nitrogen or 3.9 per cent less than the average excretion of creatinine nitrogen for the final two days of the preliminary period,(see Table IV). This decreased creatinine excretion was maintained throughout the water-period, being more pronounced in the final days of the period. The average daily decrease for the water-period was 0.033 gram of creatinine nitrogen or 5.2 per cent (Table V). The average daily decrease for the first five days of the final period was 0.013 gram of creatinine nitrogen or 2.05 per cent. The experiment ended with the daily creatinine excretion somewhat higher that at the end of the preliminary period.

In this connection data regarding the "creatinine coefficient" are of interest. This coefficient is the milligrams of creatinine-nitrogen per kilogram of body weight. As we have just seen, the creatinine excretion was decreased through the influence of the copious intake of water. This fact would tend to lower the coefficient. At the same time also the body weight was increasing thus working toward a further lowering of the coefficient. Calculating this value for the three periods we find that the coefficient of 8.89 obtained during the preliminary period was reduced to 8.36 during the water period (Table VIII). The entire final period yields a coefficient of 8.70 whereas the final day of the experiment gives us a value of 8.88 which is

practically the same as the coefficient of the preliminary period. The lower coefficient 8.70 obtained when the data of the whole period are taken into account is due to the fact that the low daily creatinine output instituted in the water period was continued during the first portion of the final period.

Excretion of Creatine: Simultaneously with the ingestion of the large quantities of water, creatine appeared in the urine. The amount of creatine excreted varied considerably on the different days, but it continued to appear on each day of copious water drinking as well as on each of the first three days of the final period. The daily excretions of creatine-nitrogen for the water period ranged from 0.024 to 0.128 grams the maximum output being registered during the final day of the increased water ingestion. The average daily excretion of creatine nitrogen for the entire water period was 0.074 gram (Table V) or 0.56 % of the total nitrogen output. On the fourth day of the final period during which the 3000 c.c. of water was eliminated from the diet, the creatine failed to appear and no trace of it could be found in the urine during the rest of the experiment.

Source of the Increased Nitrogen Excretion Accompanying Copious Water Drinking.

The appearance of creatine in the urine under the influence of copious water drinking is a most significant factor, and affords us a definite clue as to the actual source of at least a portion of the increase in the urinary nitrogen which accompanies the ingestion of excessive quantities of water. As we have already mentioned, there are two hypotheses as to the source of this in-

crease in the nitrogen output. One view asserts that the increase is due simply to a flushing-out process, thus removing the nitrogenous end-products of previously disintegrated tissues. The other view is to the effect that this increased output of nitrogen results entirely from a stimulation of protein catabolism. According to this view the nitrogen excess, instead of being composed of nitrogenous constituents which have resulted from some previous tissue cleavage and have not yet been excretedbecause the volume of fluid ingested was insufficient to afford a thorough flushing of the tissues, is due to protein catabolism promulgated through the agency of the increased water ingestion.

In all the investigations upon the influence of water drinking, made up to this time, there have never been obtained any experimental data, so far as we are aware, which place either of these hypotheses upon a firm foundation. It is evident, however, that in the present investigation our creatine data give us definite proof as to the origin, of at least a portion, of the excessive nitrogen excreted under these conditions. No urine of the preliminary period contained creatine, but as soon as the three liters of water was daily added to the diet creatine at once appeared. It is evident that the creatine appeared because of the disintegration of muscular tissue. We believe that the muscular tissue yielding the creatine could not by any chance have been disintegrated previous to the entrance of the large amount of water into the organism. We base our belief upon the fact that creatine is not a constituent of normal urine. Therefore it appears to us entirely outside the realm of possibility that each of the nitrogenous end-products of tissue catabolism should be excreted under normal conditions, except the creatine, and that the latter should remain behind and

make its appearance only when an unusual flushing occurs. The creatine was therefore derived from muscular tissue which was disintegrated under the influence of the ingestion of the excessive amounts of water. An interesting feature in this connection was the pronounced "creatine lag". An examination of Table II will reveal the fact that the bulk of the excess nitrogen of the water period was excreted on the first day of the period whereas the maximum creatine excretion occurred upon the final day of the period, and traces of the substance were still found in the urine on each of the three days following the water period. If the ingestion of the copious supply of water upon the first day of the water period caused the disintegration of sufficient muscular tissue to produce an increased output of over one gram of total nitrogen upon that day, why was not the creatine content of this same tissue excreted in the same rapid manner? An examination of the data will show us that the creatine corresponding to this tissue destruction was not excreted until a period of eight days had passed. This"creatine lag" of one week is decidedly novel so far as "lags" are concerned.

We believe that we have in the creatine data as tabulated in Table II the first definite experimental evidence in favor of the hypothesis that the increased nitrogen appearing in the urine following copious water-drinking is due to a stimulation of protein catabolism. We base our belief upon the fact that the amount of muscular disintegration necessary to produce the increase in nitrogen noted in the water period can be shown by calculation to be almost precisely the amount necessary to yield the total creatine output as determined by analysis? We arrive at this conclusion from the following calculation: Creatine contains

thirty-two per cent of nitrogen, therefore the total excretion of creatine-nitrogen (0.463 gram) is equivalent to 1.45 gram of creatine. Experiments made in this laboratory[56] have shown fresh lean beef to contain 0.446 per cent of creatine. So far as we are aware, the creatine value for human muscular tissue has not been determined. Therefore using the value determined for lean beef, a value which is probably not far removed from the actual creatine value of human muscle, we find that the excretion of 1.45 gram of creatine represents the disintegration of 325 grams of muscular tissue. Applying Voit's[57] factor (3.4%) and calculating the nitrogen value we secure 1.105 gram as the nitrogen content of the 325 grams of muscular tissue. Now if we consider the first day of the water period we see from an examination of Table II that an excess of 1.027 gram of nitrogen was excreted upon that day. On the other hand if we take into consideration the entire period during each day of which three liters of water were added to the normal diet, we observe that the excess nitrogen output was 1.079 gram. In each instance the theoretical yield of nitrogen as calculated upon the basis of the creatine output is practically duplicated by the total nitrogen value as actually determined by analysis. If the first day alone is considered the difference is 0.078 gram whereas the lower difference of 0.026 gram is obtained if the entire water period be included in the calculation.

Because of the unsettled condition of our knowledge regarding the metabolic relations of both creatine and creatinine we are well aware that many exceptions may be taken to our hypothesis as enunciated above. However, after a careful survey of all the recent hypotheses concerning the metabolic relations of these substances

we still believe our hypothesis to be the most feasible one in this connection. Of course the main objection to it is the phenomenonally protracted "creatine lag" which it embraces. It certainly is extremely unusual for a period of over one week to elapse after the tissue destruction before a certain constituent of the destroyed tissue is liberated from the organism. There is of course the possibility that the creatine enters into a secondary combination of some sort following its liberation from the muscle and that the subsequent disintegration of this secondary combination takes place gradually thus accounting for the delayed elimination of the creatine.

The observations of Lefmann[58] are of interest in connection with our creatine and creatinine data. This observer concludes that diseases of the liver or increased protein catabolism produce , first an increase, then a decrease in creatinine excretion. He further states that when the decreased creatinine output appears that there is an accompanying increase in the creatine output. Reference to Table II will show this latter point very nicely in the case of our water-drinking experiment. On the second day of the water period the creatinine output was 0.616 gram and the creatine output was 0.024. On the following day however under the influence of the added water the creatinine excretion fell to 0.589 gram and coincidently the creatine excretion increased to 0.102 gram. These conditions regarding the output of these two substances were almost exactly duplicated on the two following days i.e., we observe a creatinine output of 0.608 accompanied by a creatine output of 0.055, which are followed on the succeeding day by a decrease in creatinine to 0.589 and an accompanying increase in creatine the

output being the maximum creatine excretion of the experiment. These findings are right in line with Jefmann's observations. We did not observe any increase in the creatinine excretion at the outset of the period of increased protein catabolism, as he suggests. On the contrary the creatinine output was low throughout the water period and the normal rate of output for this urinary constituent was not assumed again until the fourth day of the final period.

Income and Outgo of Nitrogen.

The income of nitrogen was of course represented by the nitrogen content of the articles of diet, while the outgo of nitrogen was represented by the nitrogen content of the urine and feces. Data for the income and outgo of nitrogen are given in tabular form in Table VII. From the food analyses given in Table I we see that 14.654 grams of nitrogen was daily ingested. By referring to Table II it may be noted that the total excretion of nitrogen by the urine for the last two days of the preliminary period was 26.267 grams, and the excretion of nitrogen by the feces was 2.716 grams, (Table VI), thus making a total excretion of 28.983 grams for the two final days of the preliminary period. We further see that the total nitrogen ingested in the food during this same period was 29.308 grams. Thus it is very evident that 0.325 gram more nitrogen was taken into the body in the form of food material than was excreted in the form of urine and feces. The organism had therefore gained 0.325 gram of nitrogen during the preliminary period of two days, an average daily gain of 0.163 gram. While not in exact nitrogen equilibrium this condition of gaining but 0.163 gram of nitrogen daily was considered a very sat-

isfactory basis upon which to study the influence of copious water drinking, and therefore on the following day, 3000 c.c. of water was added to the daily diet, 1000 c.c. being taken during the progress of each of the three daily meals. The effect of this water ingestion, as we have observed elsewhere, was immediate and pronounced. Protein catabolism was so stimulated as to cause an increased output of urinary nitrogen during the water period amounting to 0.215 gram per day. The feces of this period, however, contained but0.973 gram of nitrogen per day as against 1.358 per day for the preliminary period. Thus more of the nitrogen of the ingested food was utilized by the body during the water period. The nitrogen balance shows a gain of 1.656 gram of nitrogen during this period of five days or a daily gain of 0.331 gram.

That the tendency for the body to store nitrogen was not limited merely to the time during which large amounts of water were being taken is shown by Table VII. During the final period of eight days, although the extra three liters of water which had been included in the daily menu of the water period, were eliminated, the tendency to store nitrogen continued. The data indicate that 7.534 grams of nitrogen were stored during an eight day interval, this being a daily gain of 0.942. The tendency to gain nitrogen decreased as the period neared its end, however and the final day showed a gain of but 0.237 gram, a value which is not far removed from the nitrogen balance of the last two days of the preliminary period. There being but 0.074 gram difference betweenthese two values it is evident that the organism, from the standpoint of its nitrogen metabolism, was in practically the same condition at the close as at the beginning of the experiment.

The point in connection with the income and outgo of nitrogen which is of the greatest practical interest is our observation of the pronounced decrease in fecal nitrogen during the period in which three liters of water were daily ingested with the meals. It seems apparent therefore that the protein constituents of the diet were more economically utilized during the period of extra water ingestion. This finding is of particular interest when considered in connection with the recent investigation of Foster and Lambert.[55] These investigators worked with dogs provided with the Pawlow pouch and demonstrated conclusively that when water was added to the diet, there was a much more copious flow of gastric juice and that this gastric juice possessed a higher acidity than did the gastric juice which was poured forth upon a similar diet minus the water. In other words water stimulated very markedly, the secretion of gastric juice. Our results here reported, finish the cycle and demonstrate the efficiency of this increased outpouring of highly acid juice. These two facts and the further observation that there was an increase in body weight, and an improvement in the general mental and physical tone of the subject are sufficiently important observations as to indicate the unquestionable benefits accompanying and following the drinking of large amounts of water with meals. We realize however, that our experiment includes data collected from but a single individual and that the general application of the principle can only be shown by further tests upon other individuals. As soon as opportunity permits we hope to experiment further along these lines. In the meantime, however, we shall consider the current statements regarding the exceedingly harmful influence of water drinking with meals as misleading.

Fecal Bacteria and Nitrogen.

The data regarding these factors are tabulated in Table IX. The method used for the quantitative determination of the fecal bacteria was a modification of Strassburger's method proposed by Dr. MacNeal of our university, as previously mentioned.

The conditions surrounding the experiment were such as to preclude the examination of the bacterial content of each individual stool. MacNeal's modification of Strassburger's method is very accurate but at the same time it is exceedingly time-consuming therefore with the large amount of chemical work necessary to be done in connection with this investigation and others running coincidently we were unable to make frequent bacterial examinations. The data therefore, as tabulated in Table IX represent the results form three stools, one being examined during the preliminary period, one during the water period and one during the final period. It is our purpose to study this feature farther in the near future, and make a bacterial examination of each individual stool.

Regarding the excretion of bacteria in the feces our data show that the daily output of dry bacteria was decreased under the influence of the copious water drinking. This fact is clearly shown by a daily excretion of 5.327 grams of dry bacteria during the preliminary period as compared with an excretion of 4.579 grams per day during the water period. This satisfactory change in the content of intestinal bacteria was further accentuated in the final period in which the bacteria value was reduced to an excretion of only 3.273 grams per day. The bacteria constituted 20.88 % of the dry feces during the preliminary period, the much higher value of 24.37 % during the water period and then assumed practically the

original value (21.23 %) in the final part of the experiment. The pure bacteria as isolated contained very similar nitrogen values.

Some interesting and very practical conclusions may be drawn from the data on fecal nitrogen. The data indicate that the daily output of nitrogen by way of the feces was 1.358 grams during the preliminary period, and that under the influence of the daily ingestion of three liters of water this value was reduced to 0.973. Apparently then the ingestion of this large amount of water caused a more economical utilization of the protein constituents of the diet. However when we consider our bacteria data in connection with our total nitrogen data we are much more impressed with the efficiency of the water diet. In the case of the preliminary period, for example, by subtracting the bacterial nitrogen from the total nitrogen we note that 0.774 gram of nitrogen of non-bacterial origin was excreted daily. This value was reduced to 0.497 gram for the water period. There seems to us but one explanation for this condition of affairs. Evidently the water has brought about conditions which have caused a more rapid digestion and absorption of the protein constituents of the diet. This is borne out by the data showing a decrease form 0.774 to 0.497 under the influence of the water. There being less nitrogenous material present in the intestine during the water period naturally the bacteria could not develop as satisfactorily as during the preliminary period. ~~for example, as during the normal period.~~ Therefore we would expect to have less bacterial nitrogen excreted daily, an expectation which is fully verified by our data, which show 0.584 gram of this form of nitrogen to have been excreted daily during the preliminary period as against an excretion of 0.476 gram per day for the water

period.

In the final period of the experiment the conditions in some respects resemble those of the preliminary period while in other respects they are more nearly like those in force during the water period. For example the excretion of total nitrogen was very similar to that of the preliminary period being 1.257 grams per day as compared with the daily excretion of 1.358 for the preliminary period. At the same time, however, the daily output of bacteria was very low, in fact lower than the output of the water period and constituting only about 60 per cent of the daily output of the preliminary period. We are somewhat skeptical as to whether this stool was a representative one, inasmuch as at the stage in the experiment during which the stool was collected the subject was under a rather severe nervous strain due to the illness of his wife and coupled with a troublesome attack of neuralgia. In fact the general conditions surrounding the subject at this particular time were such as might easily have caused nervous disorders sufficient to have upset the metabolic rhythm of his organism. Certainly it is an unexpected finding to secure such fecal variations as those observed in the final period, as compared with the preliminary period, when we consider that the diet was absolutly uniform from day to day. This point will be further investigated.

Conclusions.

The daily drinking of three liters of water with meals, for a period of five days, by a man 22 years of age who was in a condition of nitrogen equilibrium through the ingestion of a uniform diet, was productive of the following findings:-

1) An increase in body weight, aggregating about two pounds.
2) A decreased excretion of creatinine and the coincident appearance of creatine in the urine.
3) An increased excretion of urinary nitrogen, the excess nitrogen being mainly in the form of urea, ammonia and creatine.
4) The increased excretion of urinary nitrogen under the influence of copious water-drinking is due, in the main, to the stimulation of protein catabolism. This is clearly indicated, in the present experiment, inasmuch as the creatine output represents the disintegration of sufficient muscular tissue to account for the entire increase in the excretion of total nitrogen.
5) A decreased excretion of fecal nitrogen of sufficient magnitude to secure a lowered excretion of both the bacterial and the non-bacterial nitrogen.
6) A decrease in the quantity of bacteria excreted daily.
7) An increase in the precentage of the total nitrogen appearing as bacterial nitrogen.
8) A lower "creatinine coefficient".
9) A more economical utilization of the protein constituents of the diet.
10) No marked alteration in the number or character of the blood cells.

Table I.

Diet: Amount and Composition.

	Crackers gms	Corn-flakes gms	Peanut-butter gms	Butter gms	Sugar gms	Milk gms	Water c.cm
Percentage of Nitrogen	1.510	1.044	4.493	0.076	——	0.549	——
Amt ingested daily during Prelim. and Final Periods	300	75	60	75	36	1200	900
Amt ingested daily during Water Period	300	75	60	75	36	1200	3900

Table Ia.

Body Weights; Volumes and Sp. Gr. of the Urines.

Date	Day of Experiment	Body weight of subject	Urine	
			Volume	Sp. Gr.
Preliminary Period.				
		kilos	cu. cms	
3-3-'09	1	71.69	945.0	1.024
3-4-'09	2	70.82	740.0	1.030
3-5-'09	3	70.23	700.0	1.030
3-6-'09	4	70.63	830.0	1.030
3-7-'09	5	71.09	920.0	1.0275
3-8-'09	6	71.31	880.0	1.028
Water Period.				
3-9-'09	7	71.42	3440.0	1.008
3-10-'09	8	71.94	3840.0	1.006
3-11-'09	9	72.00	3670.0	1.007
3-12-'09	10	72.26	3610.0	1.0075
3-13-'09	11	72.18	4020.0	1.007
Final Period.				
3-14-'09	12	72.17	1590.0	1.016
3-15-'09	13	72.06	885.0	1.025
3-16-'09	14	72.21	1025.0	1.025
3-17-'09	15	72.24	890.0	1.026
3-18-'09	16	72.15	830.0	1.027
3-19-'09	17	72.11	870.0	1.027
3-20-'09	18	72.29	930.0	1.0265
3-21-'09	19	72.21	1480.0	1.020

Table II

Nitrogen Partition.

Date	Experimental Day	Total Nitrogen	Urea	Ammonia	Creatinine	Creatine
Preliminary Period						
		grams	grams	grams	grams	grams
Mar. 3, '09	1	11.981	10.401	0.406	0.623	
Mar. 4, '09	2	11.323	9.353	0.345	0.642	
Mar. 5, '09	3	12.057	10.375	0.357	0.624	
Mar. 6, '09	4	12.987	11.358	0.288	0.629	
Mar. 7, '09	5	13.084	11.476	0.305	0.619	
Mar. 8, '09	6	13.183	11.568	0.369	0.651	
Water Period						
Mar. 9, '09	7	14.161	12.596	0.486	0.610	0.063
Mar. 10, '09	8	13.491	11.583	0.499	0.616	0.024
Mar. 11, '09	9	12.981	10.667	1.098	0.589	0.102
Mar. 12, '09	10	12.976	11.455	0.485	0.608	0.055
Mar. 13, '09	11	13.138	12.060	0.275	0.589	0.128
Final Period						
Mar. 14, '09	12	11.823	9.952	0.810	0.602	0.041
Mar. 15, '09	13	11.836	10.198	0.422	0.639	0.015
Mar. 16, '09	14	12.925	11.169	0.369	0.586	0.035
Mar. 17, '09	15	11.946	10.299	0.374	0.642	
Mar. 18, '09	16	12.017	10.374	0.333	0.629	
Mar. 19, '09	17	13.030	10.835	0.344	0.659	
Mar. 20, '09	18	12.905	11.293	0.325	0.627	
Mar. 21, '09	19	13.160	11.452	0.399	0.641	

Table III

Percentage of Total Nitrogen in the form of Urea, Ammonia, Creatinine and Creatine.

Date	Experimental Day.	Nitrogen	Urea	Ammonia	Creatinine	Creatine
Preliminary Period						
Mar. 3, '09	1	100	86.81	3.39	5.20	
Mar. 4, '09	2	100	82.60	3.05	5.67	
Mar. 5, '09	3	100	86.05	3.03	5.17	
Mar. 6, '09	4	100	87.46	2.22	4.84	
Mar. 7, '09	5	100	87.71	2.33	4.73	
Mar. 8, '09	6	100	87.75	2.80	4.83	
Water Period						
Mar. 9, '09	7	100	88.95	3.43	4.31	0.44
Mar. 10, '09	8	100	85.86	3.70	4.57	0.18
Mar. 11, '09	9	100	82.17	8.46	4.54	0.79
Mar. 12, '09	10	100	88.49	3.74	4.69	0.42
Mar. 13, '09	11	100	91.79	2.09	4.48	0.97
Final Period						
Mar. 14, '09	12	100	84.17	6.85	5.10	0.35
Mar. 15, '09	13	100	86.16	3.57	5.40	0.13
Mar. 16, '09	14	100	86.41	2.85	4.53	0.27
Mar. 17, '09	15	100	86.23	3.13	5.37	
Mar. 18, '09	16	100	86.33	2.77	5.23	
Mar. 19, '09	17	100	83.16	2.64	5.58	
Mar. 20, '09	18	100	87.51	2.52	4.86	
Mar 21, '09	19	100	87.02	3.03	4.87	

Table IV.

Increased Nitrogen Excretion of the First Day of the Water Period as Compared with the Average of the Last Two Days of the Preliminary Period. (Urine)

	Total Nitrogen	Urea	Ammonia	Creatinine	Creatine
Increase in Grams	1.027	1.074	0.149	-0.025	0.063
Percentage Increase	7.82	9.32	44.21	-3.94	
Percent increase as compared to increase of total Nitrogen.	100.0	104.67	14.52	-2.43	6.13

Table V.

Average Daily Increase in the Nitrogen Excretion of the Water Period as compared with the Last Two Days of the Preliminary Period. (Urine)

	Total Nitrogen	Urea	Ammonia	Creatinine	Creatine
Increase in Grams	0.215	0.150	0.232	-0.033	0.074
Percentage Increase	1.64	1.30	68.84	-5.20	
Percent Increase as compared to increase of total Nitrogen	100.0	69.77	107.91	-15.35	34.42

Table VI

Weight and Nitrogen Content of Feces.

Period	Length of Period	Fresh Feces.	
		Weight	Nitrogen
		grams	grams
Preliminary	6	630.1	8.147
Water	5	370.1	4.867
Final	8	783.9	10.056

Table VII

Income and Outgo of Nitrogen.

Period	Length of Period	Income	Outgo		Gain or Loss	Average Gain or Loss per day
		Food	Urine	Feces		
Preliminary	2	29.308	26.267	2.716	+0.325	+0.163
Water	5	73.270	66.747	4.867	+1.656	+0.331
Final	8	117.232	99.642	10.056	+7.534	+0.942
Final Period Last day	1	14.654	13.160	1.257	+0237	+0.237

Table VIII

Creatinine Coefficient.

Period	Creatinine mgs	Body Weight Kilos	Creatinine Coefficient
Preliminary	631.0	70.96	8.89
Water	602.0	71.96	8.36
Final	620.0	72.17	8.70
Last day of Final Period.	641.0	72.21	8.88

Table IX.

Fecal Bacteria and Nitrogen.

Period.	Fecal Bacteria			Fecal Nitrogen.				
	Weight	Percent of Dry Feces	Nitrogen	Total Weight	Bacterial		Non-bacterial	
					Weight	Percent	Weight	Percent
	Grams per Day		Per Cent.	Grams per Day	Grams		Grams	
Preliminary	5.327	20.88	10.97	1.358	0.584	42.9	0.774	57.1
Water	4.579	24.37	10.40	0.973	0.476	49.1	0.497	50.9
Final	3.273	21.23	11.54	1.257	0.378	30.1	0.879	69.9

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